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Human unrestricted somatic stem cells ameliorate sepsis-related acute lung injury in mice

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Abstract

Background Aims: Sepsis and related disorders, especially acute lung injury (ALI), are the most challenging life-threatening diseases in the hospital intensive care unit. Complex pathophysiology, unbalanced immune condition, and high rate of mortality complicate the treatment of sepsis. Recently, cell therapy has been introduced as a promising option to recover the sepsis symptoms. The aim of this study was to investigate the therapeutic potential of human unrestricted somatic stem cells (USSCs) isolated from human umbilical cord blood in the mouse model of ALI. USSCs significantly enhanced the survival rate of mice suffering from ALI and suppressed concentrations of proinflammatory mediators TNF-α, and interleukin (IL)-6, and the level of anti-inflammatory cytokine IL-10. ALI mice injected by USSCs showed notable reduction in lung and liver injury, pulmonary edema, and hepatic enzymes, compared with the control group. These results determined the in vivo immunomodulatory effect of USSCs for recovery of immune balance and reduction of tissue injury in the mouse model of ALI. Therefore, USSCs can be a suitable therapeutic approach to manage sepsis disease through the anti-inflammatory potential.

KEYWORDS
acute lung injury, cell therapy, inflammation, umbilical cord, unrestricted somatic stem cells

1 | INTRODUCTION

Sepsis is defined as systemic inflammation followed by an intensive bacterial or fungal infection and is associated with a high rate of mortality (Mayr, Yende, & Angus, 2014). Hyperactivation of innate and acquired immune systems against pathogens leads to cytokine storm and, ultimately, multiorgan injury (van der Poll & Opal, 2008). Lung failure is the most frequent challenge related to sepsis and leads to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Lipopolysaccharides (LPS), endotoxins derived from gram-negative

bacteria, are the most potent factor to induce ALI in humans and animals. LPS induces production of different kind of cytokines, such as TNF-α, interleukin (IL)-1b, IL-6, IL-10, and IL-12 in both human and murine monocytes, which may trigger extensive inflammatory responses (Rossol et al., 2011; H. M. Wang, Bodenstein, & Markstaller, 2008). Although new evidence suggests that the mortality rate of ALI/ADRS has declined recently, it still remains about 26% in the USA (Erickson, Martin, Davis, Matthay, & Eisner, 2009). The current guidelines for the management of sepsis recommend procedures like antibiotic therapy, fluid therapy, and administration of blood products in the early stages of the disease (Rhodes et al., 2017). The current standard treatment of sepsis will not be liable in the near future because of factors like the advent of antibiotic-resistant microbes; so, a new therapeutic approach is required to overcome this challenge (Grimaldi & Vincent, 2017). Cell therapy, as an alternative therapeutic option with a live-cell formulation, displays significant outcomes in inflammatory diseases. Selection of suitable cell candidates for a therapeutic approach is the most challenging issue in the cell-based therapy era. Recent studies have determined that mesenchymal stromal cells (MSCs) can be efficient on sepsis and related disorders due to immunomodulatory effects. In fact, MSCs can improve immune balance in sepsis patients by regulation of immune cell function, which is very significant in the management of inflammatory condition like ALI/ADRS (Johnson, Soeder, & Dahlke, 2017). MSCs isolated from different types of tissues, such as bone marrow (Krasnodembskaya et al., 2012), adipose tissue (Elman, Li, Wang, Gimble, & Parekkadan, 2014), umbilical cord (Li, Li, Liu, Tang, & Wei, 2012), and Wharton's jelly (Cóndor et al., 2016), have been studied in the mouse model of sepsis. Administration of MSCs results in modulation of cytokines, improvement of organ injuries, and elevation of survival rate. On the other hand, noninvasive isolation and access to appropriate cell count for infusion are the concerns of stem cell therapy.

Human unrestricted somatic stem cell (USSC) is a nearly new cell type isolated from umbilical cord blood for the first time in 2004 by Kogler and colleagues. These cells are called pluripotent or MSCs progenitor because of their higher proliferative activity and the wide range of differentiation capacity. USSCs can expand up to 10^{15} cells without deviation in normal karyotype and emersion of cell senescence (Kögler et al., 2004). Significantly, differentiation of USSCs to various cell types of all three germ layers is determined in both in vitro and in vivo studies (Wernet, Trapp, Zweigerdt, Mann, & Trompeter, 2010). A low immunogenic nature similar to MSCs is an appropriate property of USSC, which paves the path for cell therapy applications. The therapeutic effect of these cells has been investigated in different preclinical studies that provided promising consequences. Transplantation of USSCs to the myocardial infarction (MI) animal model improves left ventricle function due to secretion of trophic factors and regulation of cytokines (Rabald et al., 2008). Intrahepatic injection of USSCs in the murine model of epidermolysis bullosa is followed by cell migration to blistering sites and recovery of injuries occurrs in response to secretion of type VII collagen (Liao et al., 2015). Regulation of cellular immunity, as the more considerable property of USSCs, is provided by secretion of different cytokines. Mixed lymphocyte reaction test in the presence of USSCs has clarified the immunomodulatory effect of these cells against different HLA classes and gene polymorphisms (Ebrahimi et al., 2010). So, USSC may be a valuable source for ALI cell therapy due to ease of availability, noninvasive processing, significant proliferation potential, acceptable immunomodulatory, and nonimmunogenic traits.

In the present study, we investigated the therapeutic potential of USSCs in the mouse model of ALI to evaluate the immunomodulatory effect of transplanted cells on the systemic inflammation induced by LPS. Moreover, the tissue protective capacity of USSCs was assessed on the lung and liver damage caused by widespread inflammatory responses.

2 | MATERIALS AND METHODS

2.1 | Animal care

Male C57BL/6 mice (8–12 weeks old, weighing 22–30 g) were purchased from the Tehran Laboratory Animal Center, Tehran, Iran. All mice were housed in the animal facility at the Tehran University Laboratory Animal Center according to the NIH animal care guidelines. All animal experimental procedures were approved by the Animal Care Committee of the Tehran University of Medical Sciences.

2.2 | Isolation and expansion of USSCs

Collection of umbilical cord blood was performed after mothers' informed consent according to the approved ethical guidelines of the Tehran University of medical sciences. USSCs were isolated and cultured according to Kogler et al. (2004)'s procedure with minor modifications. Briefly, mononuclear cells were fractionated from cord blood first by hydroxyethyl starch buffer (Sigma-Aldrich, St. Louis, MI) followed by ficoll (Panbiotech, Germany) density gradient. After the lysis of RBCs, $5–7 \times 10^6$ isolated cells were counted and cultured in T25 flasks with the specific medium, including DMEM low glucose (Gibco-BRL, Grand Island, New York) supplemented with 30% FBS, dexamethasone 10^{-7} M (Sigma-Aldrich), 2 mM glutamine (Gibco), 100 U/ml streptomycin (Gibco), and 100 mg/ml penicillin (Gibco). Cell incubation was prepared at 37°C in 5% CO_2. USSC colonies were appeared 5–10 days later and passaged with 0.25% trypsin. The cells were expanded in the same medium supplemented with 10% FBS without dexamethasone.

2.3 | Analysis of surface markers

To confirm the identity of cells, surface markers including CD105, CD90, CD73, CD31, CD34, CD45, and HLA-DR were analyzed by flow cytometry (BD FACSCalibur, BD Biosciences, Qume Drive, San Jose).

2.4 | In vitro differentiation

To induce osteocyte differentiation, USSC cells were cultured in specific medium containing DMEM (Gibco), 10% FBS (Gibco), 10 mM β-glycerol phosphate (Sigma-Aldrich), 10^{-7} M dexamethasone, and

50 μg/ml ascorbic acid biphosphate (Sigma-Aldrich) for 21 days. Alizarin Red (Sigma-Aldrich) staining was served to confirm calcium deposits.

Adipocyte differentiation was determined by Oil Red O staining followed by 21-day culture of USSC in DMEM supplemented with 10% FBS, 250 nM dexamethasone, 60 nM insulin, 0.5 mM isobutyl-methylxanthine, 0.2 mM and indomethacin (all from Sigma-Aldrich).

For detection of DLK-1 via immunocytochemistry, the cultured cells were washed and fixed by 4% (v/v) paraformaldehyde (Sigma-Aldrich) in PBS for 20 min. After blocking with 10% goat serum for 1 hr, the cells were incubated with DLK-1 primary antibody (Abcam). The samples were washed three times with PBS followed by 1-hr incubation with a secondary antibody in room temperature. The nuclei were stained by 4′, 6-diamidino-2-phenylindole (Sigma-Aldrich).

2.5 | LPS-induced sepsis and experimental design

LPS from *Escherichia coli* o55:25 (L2880; Sigma-Aldrich) was liquefied in phosphate buffer saline with 1 mg/ml concentration. To induce endotoxemia, the mice were injected intraperitoneally by 400 μg/mouse dilution and randomly assigned in one of three groups: (a) LPS group, (b) LPS+USSC group, and LPS+PBS group ($n = 6$ for each group). A total of 1×10^6 USSC cells were washed and resuspended in 200 μl PBS. Two hours after the LPS injection, the mice received 1×10^6 USSC cells or PBS by injection in the tail vein. The animals were killed 24 hr after USSC or PBS treatment of cytokine measurement, tissue histopathology, and biochemical analysis. For survival study, three groups of mice ($n = 8$) were monitored every 12 hr for 7 days.

2.6 | Lung and liver histopathology

Lung and liver were harvested from each examined group 24 hr after treatment. After fixation with 10% formalin (Merck, Germany), the samples were embedded in paraffin and cut into sections with a thickness of 5 μm. Hematoxylin and eosin-stained samples were analyzed by a veterinary pathologist to evaluate tissue injury and inflammation.

2.7 | Wet-dry analysis

Harvested lungs from all tested groups ($n = 3$) were placed into microcentrifuge tubes and weighted. After overnight desiccating in a 85°C oven, the lungs were weighted again. The wet-dry ratio was assessed by dividing the weight of wet lung into the weight of dry ones.

2.8 | Measurements of serum cytokines

For detection of cytokines, mouse blood samples were obtained by cardiac puncture and incubated in room temperature for 20 min to clot. After centrifugation at 400 g for 5 min, the serum was collected and stored at −80 °C. The serum levels of IL-6 (ab100712; Abcam, UK), IL-10 (M1000B; R&D systems) and TNF-α (ab208348; Abcam, UK) were measured by ELISA according to the manufacturer's protocol. All samples were run in triplicate, and the absorbance of each sample was measured by ELISA reader (ELx800^{TM}, BioTek Instruments, Italy) at 450 nm.

2.9 | Blood biochemical analysis

The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by (selectra ProM, ELITech Group, France) according to the manufacturer's instruction.

2.10 | Statistical analysis

All data are expressed as the mean ± standard error of the mean. Statistical analysis of data was accomplished by using the GraphPad Prism 6 software. Differences between groups were evaluated with one-way analysis followed by Newman-Keuls posttest. The survival rate of mice was analyzed by the Caplan–Meier method, and group compression was assessed via the Mantel–Cox log-rank test. The statistical significance for differences between values was accepted at $p < 0.05$.

3 | RESULTS

3.1 | Characterization and immunophenotyping of USSCs

Fourteen days after isolation, the adherent colon of USSCs was observed, which appeared in single spindle shape morphology after the first passage (Figure 1a,b). Analysis of immunophenotyping by flow cytometry revealed that the cells were positive for CD90, CD73, and CD105 but negative for CD45, CD34, CD31, and HLA-DR (Figure 1c). Osteogenic differentiation of the cells at passage 4 was confirmed after 21 days culture in specific media followed by Alizarin red staining (Figure 1d). Differentiation of USSCs to adipogenic lineage after 21-day cell culture in adipo-induction medium was not proved by Oil red staining (Figure 1e); therefore the cells were immunostained by the DLK-1 antibody as a specific marker for characterization of USSCs (Figure 1f; Figure 2)

3.2 | USSCs treatment attenuates systemic inflammatory responses

TNF-α and IL-6 are the main proinflammatory cytokines that play a critical role in the progression of sepsis (Ulloa & Tracey, 2005). On the other hand, the serum level of IL-10 as an anti-inflammatory cytokine increase followed by LPS injection. Therefore, we studied the amount of the above cytokines 24 hr after cell or saline treatment (Figure 3). LPS notably enhanced the serum concentration of TNF-α, IL-6, and IL-10 in the reference time interval ($p < 0.01$, $p < 0.001$, $p < 0.0001$, respectively). Although PBS treatment was not effective on the serum levels of cytokines, the administration of USSCs significantly declined the abundance of both pro- and anti-inflammatory cytokines close to

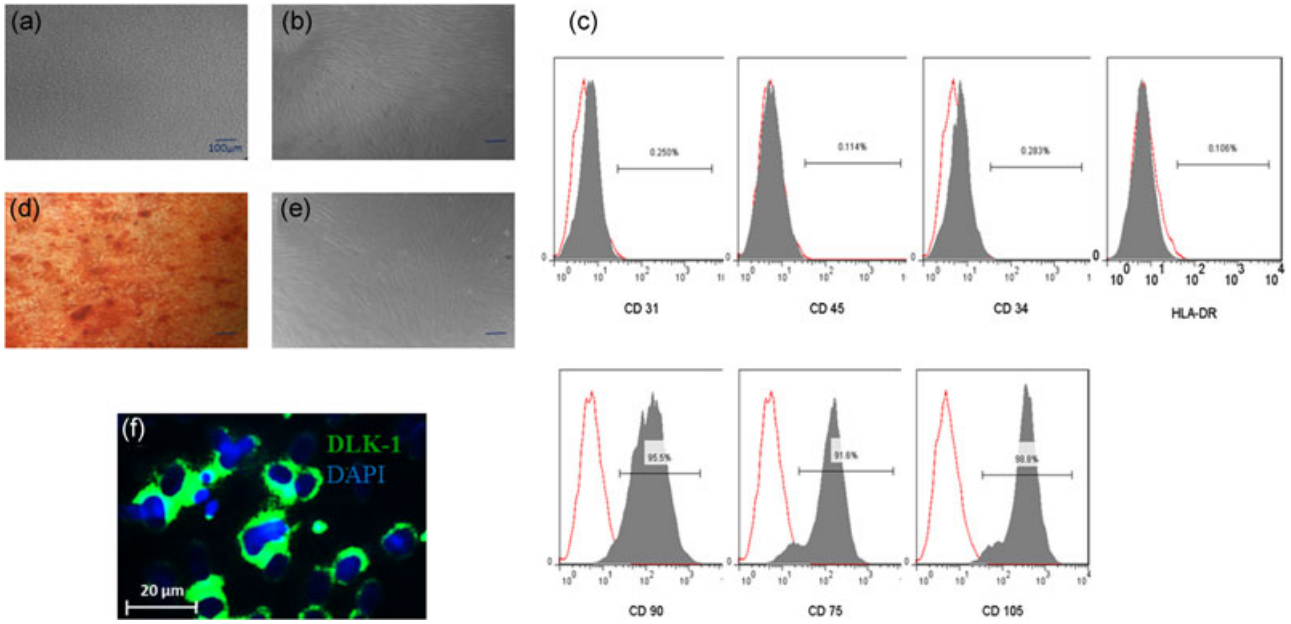

FIGURE 1 Characterization of isolated USSCs. (a) USSC colony appeared after 2 weeks of isolation and (b) USSCs with high confluency (magnification 100×). (c) Immunophenotyping of USSCs showed that the cells are positive CD90, CD73, CD105, and negative for CD45, CD34, CD31, and HLA-DR markers. Differentiation pattern of USSC (d) to osteocyte that showed by Alizarin red staining and (e) to adipocyte lineage that not proved by Oil red O staining (magnification 100×). (f) Immunocytochemistry of USSCs with DLK-1 antibody (green), the nucleus of cells was stained by DAPI (blue). DAPI: 4′,6-diamidino-2-phenylindole; USSC: unrestricted somatic stem cells [Color figure can be viewed at wileyonlinelibrary.com]

control counterparts ($p < 0.01$ for TNF-α, IL-6, and $p < 0.001$ for IL-10). These results suggest that USSCs can efficiently modulate systemic immune responses induced by LPS.

3.3 | USSCs prevents severe lung and liver injury

Twenty-four hours after LPS injection, severe hemorrhage, associated with hemosiderin, fibrosis, and alveolar thickening, was observed. Histological analysis of LPS-induced liver tissue showed severe necrosis of hepatocytes, and the central vein became dilated and congested. Moreover, intensive infiltration of neutrophils occurred in both lung and liver sections. Saline injection after LPS intervention also displayed hepatocyte necrosis, lung edema, alveolar fibrosis, and increase in inflammatory cells. Although USSC treatment of ALI mice was associated with moderate injuries, the intensity of fibrosis, pulmonary edema, and neutrophil infiltration was significantly decreased, as well as a regenerative foci of hepatocytes in the response of mild necrosis was detected in liver tissue (Figure 3).

3.4 | USSCs treatment enhances ALI survival rate

Administration of cells or PBS was performed 2 hour after injection of LPS. The mice were followed up every 12-hr for 7 days to evaluate the survival rate of each group. The Caplan–Meir curve showed 25% (2 of 8 mice surviving until 60 hr) after LPS intervention, whereas USSCs treatment improved survival rate to 87.5% (7/8) compared with the LPS group ($p = 0.004$). However, intravenous infusion of saline did not significantly alter mortality compared with the

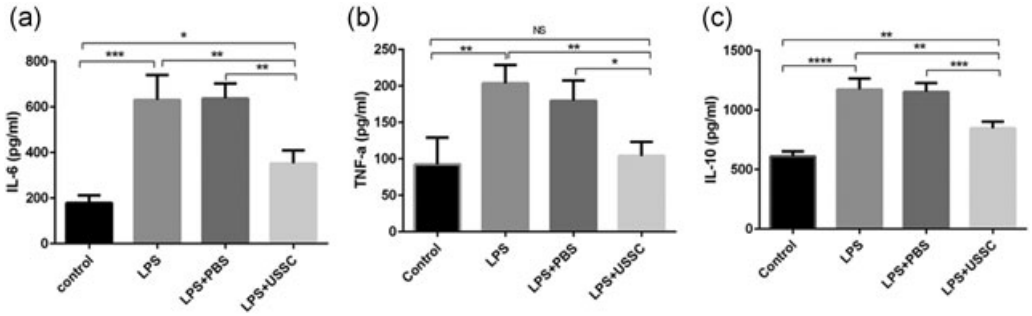

FIGURE 2 Administration of USSCs balances the systemic inflammation caused by LPS. LPS intervention resulted in increasing concentrations of (a) IL-6 and (b) TNF-α as the proinflammatory agents and (c) IL-10 as a major anti-inflammatory cytokine. Intravenous injection of USSCs effectively reduces the serum level of above cytokines. $n = 3$ per group. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$ and $^{****}p < 0.0001$. LPS: lipopolysaccharides; USSCs: unrestricted somatic stem cells

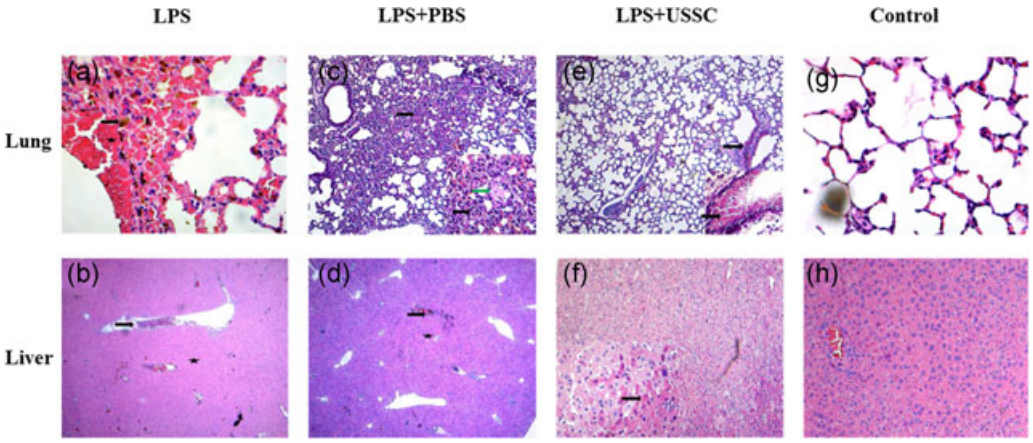

FIGURE 3 USSCs treatment rescue the lung and liver sever injury in mice model of ALI. In the LPS group, severe hemorrhage (star) associated with hemosiderin (black arrow), neutrophil infiltration (green arrow), and fibrosis causing alveolar wall thickness (arrowhead) in the lung biopsy and severe necrosis of hepatocytes (star), neutrophil infiltration (black) in the liver. USSC treatment reduced infiltration of inflammatory cells (green arrow), lung fibrosis and hepatocyte necrosis (arrow). Magnifications: (a) and (g) ×100, (b) ×10, (d) ×20, (c), (e), (f), and (h) are ×40, all inlets in the corner of (c), (e), and (f) are ×100. ALI: acute lung injury; LPS: lipopolysaccharides; USSC: unrestricted somatic stem cells [Color figure can be viewed at wileyonlinelibrary.com]

untreated group (3 of 8 mice remained with 37.5% survival rate) but the comparison of this group with USSC group was statistically considered ($p = 0.017$; Figure 4).

3.5 | USSCs treatment balance liver enzymes

Liver enzymes AST and ALT release in response to hepatocellular injury and are considered liver dysfunction indicators. The serum amount of AST and ALT was significantly increased in the mice model as a result of LPS injection compared with the control group ($p < 0.001$ and $p < 0.01$, respectively). Figure 5a,b represent that tail vein administration of USSCs effectively declined the concentration of liver enzymes (ALT 106.3 ± 5.13 U/L, $p < 0.05$ and AST 270.3 ± 8.35 U/L, $p < 0.05$) in comparison with the saline group (ALT 144 ± 8.14 U/L and AST 338 ± 13.6 U/L). Consequently, USSCs contributed to liver function recovery by presenting this hepatic protective effect.

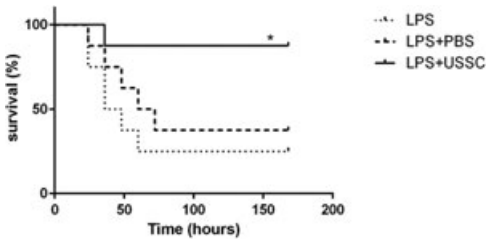

FIGURE 4 The survival rate of LPS-induced mice improved after treatment of USSCs. Caplan–Meir curve showed the percentage of surviving mice in LPS injected ($n = 8$), LPS+PBS infused ($n = 8$) and USSCs treated group ($n = 8$). USSCs significantly improve the survival rate of ALI-suffering mice compared with the untreated group (87/5% vs 25%; $p = 0.004$). ALI: acute lung injury; LPS: lipopolysaccharides; PBS: phosphate-buffered saline; USSC: unrestricted somatic stem cells

3.6 | USSCs treatment alleviates lung edema

The state of pulmonary edema, which was scored by wet/dry ratios of lungs, was improved after 24-hr injection of LPS ($p < 0.0001$). The mice model that received USSCs showed a remarkable reduction in wet/ dry weight ratio versus LPS and saline groups ($p < 0.001$ and $p < 0.01$, respectively; Figure 5c).

4 | DISCUSSION

In this study, USSC was successfully isolated from umbilical cord blood and characterized based on the pattern of CD markers, osteocyte determination, no adipogenic differentiation capacity, and DLK-1 expression. These cells were used as a new cell therapy approach in the ALI mouse model. The result of this study determine that intravenous administration of USSC 2 hr after LPS-endotoxemia induction notably upgraded survival rate, clearly recovered the systemic and pulmonary inflammatory responses, and improved the histopathological state of liver and lung injury.

Sepsis is known as the systemic inflammatory response syndrome (SIRS), which is characterized by cytokine release, severe tissue injury, and ischemia-reperfusion (Angus & van der Poll, 2013). Lungs are the most susceptible organ and the origin of sepsis inflammatory condition that finally appears as respiratory diseases like ALI and ARDS (Hostiuc, Dermengiu, Ceausu, Rusu, & Curca, 2011). Bacterial toxins, especially endotoxins, are certainly the most considerable factor in the development of sepsis. LPS is the most important component of the gram-negative bacteria that triggers intense inflammation in both human and animal models (Copeland, Warren, Lowry, Calvano, & Remick, 2005; Ramachandran, 2014). Intraperitoneal injection of LPS in mice results in intensive secretion of cytokines, alteration of respiratory function, induction of pulmonary

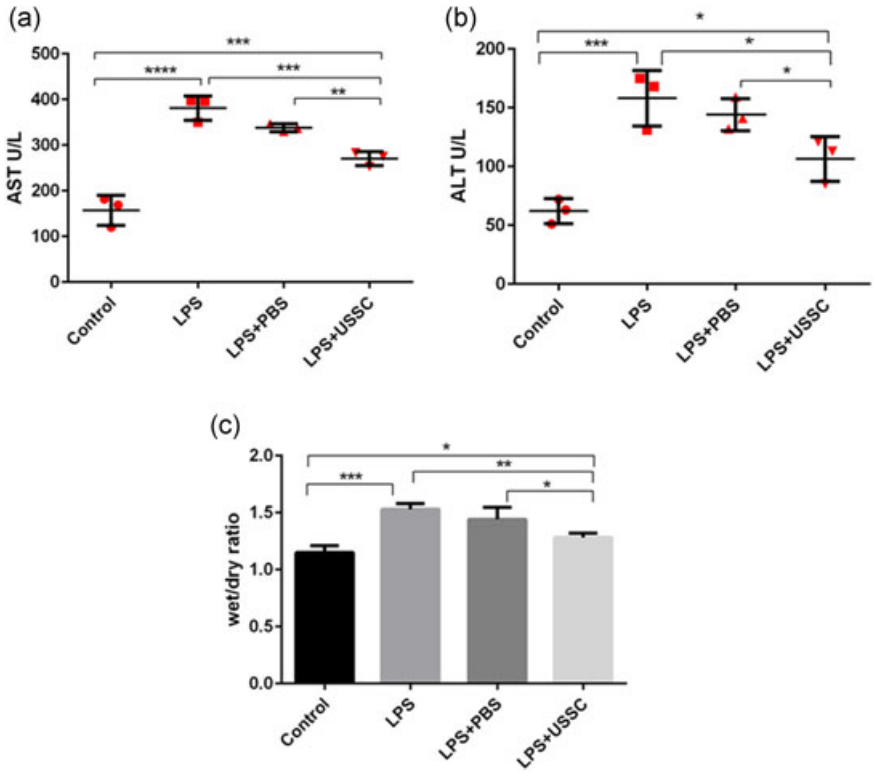

FIGURE 5 USSCs alleviate high level of hepatic enzymes and pulmonary edema induced by LPS. (a,b) The serum levels of liver biomarkers, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were evaluated in each exanimated group. Cell treated mice represent a lower dose of AST and ALT compared with the LPS group ($p < 0.05$). (c) Lung edema was measured by wet/dry weight ratio. $n = 3$ per group. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$. LPS: lipopolysaccharides; USSC: unrestricted somatic stem cells [Color figure can be viewed at wileyonlinelibrary.com]

edema, and accumulation of inflammatory cell (Rojas, Woods, Mora, Xu, & Brigham, 2005). Hence, we prepared the mouse model of ALI-related sepsis by administration of LPS to investigate the therapeutic effects of USSC cells as a cell therapy candidate.

Recently, several preclinical studies reported the anti-inflammatory and immune regulatory effects of MSCs on different type of sepsis animal models. Bone marrow and adipose tissue-derived MSCs served as the new therapeutic option in sepsis clinical trials (Wilson et al., 2015; Zheng et al., 2014). Because cell-based therapy may efficiently manage sepsis-related disorders, plenteous studies have been performed to introduce a suitable cell source. Ease availability, noninvasive isolation method, low immunogenicity, and high-proliferative potential are the main features that must be considered to access desired consequences. Furthermore, for successful cellular therapy in the sepsis era, it is necessary to modulate enhanced inflammatory responses, decrease organ injuries, and decline mortality. MSC derived from different sources is studied as a cell therapy candidate in sepsis and ALI animal models because of its remarkable immunomodulatory and regenerative effects (Johnson et al., 2017). Despite suitable characteristics of MSCs, some challenges remain insoluble like low proliferation rate and precocious senescence. USSC cells, which are derived from umbilical cord blood with high-proliferative potential and a wide range of differentiation capability, share some MSC traits like low immunogenicity and immunomodulatory properties. Differentiation capacity of USSCs to various type of cells with different embryonic origin is determined in both in vitro and in vivo experiments. Regenerative and therapeutic quality of USSCs is studied in several preclinical models including MI (Iwasaki et al., 2009; Kim et al., 2005), bone healing (Handschel et al., 2010; Jäger et al., 2007), and spinal cord injury (Schira et al., 2012). Cell-based therapy for the murine model of wound healing and epidermolysis bullosa was also determined via secretion of type VII collagen by USSCs (Liao et al., 2015). In the case of infectious diseases, there has been no report of USSC treatment in preclinical experiments. Given the significant possibility of immunomodulation and regeneration, USSC can be introduced as a new cell-based option for ALI and sepsis management.

LPS induction activates the TLR4-related signaling pathways, which finally results in increasing proinflammatory cytokines. On the other side, the production of anti-inflammatory cytokines like IL-10 also occurs by LPS induction. The recovery of the balance between pro- and anti-mediators is the major purpose of sepsis management (H. D. Wang, Lu, & Qi, 2009). IL-6 and TNF-α are two main proinflammatory cytokines that enhance rapidly after LPS inoculation (Rojas et al., 2005). In the present study, in spite of the intense increase in the serum level of IL-6 and TNF-α after LPS injection, USSC treatment effectively reverts the amount of this component close to the normal state. Hence, USSC therapy, as a new option for sepsis management, could mediate the remission of cytokine storm.

The controversial role of IL-10 in the systemic inflammation is not completely understood because of some adverse effects in septic patients and different outcomes of animal models. In human sepsis, the elevated concentration of IL-10 is associated with a higher risk of infection maintenance, organ injury, and death. IL-10 is associated with the development of sepsis in trauma patients (Giannoudis et al., 2000). Moreover, prolongation of the expression of anti-inflammatory cytokines leads to the appearance of a critical state termed compensatory

anti-inflammatory response syndrome (CARS) and finally deactivation of the immune system (Ward, Casserly, & Ayala, 2008). Therefore, IL-10 needs to sustain in the rational enhanced level, which suppresses inflammatory responses but does not trigger CARS. The enhancement of IL-10 level to approximately two folds observed after LPS intervention is in the agreement with other reports (Hu et al., 2016; Li et al., 2012). Administration of USSC could decline the rebellious amount of IL-10, but in the compression with the normal state, the serum level of IL-10 was still elevated. This event seems beneficial to support the balance of immune system reactions in the inflammatory condition.

The experimental results determined that USSCs treatment improved the survival of ALI-suffering mice probably raised from the suppression of inflammatory responses and regenerative effects. Lungs are the most vulnerable organ in septic patients with a notably poor outcome. Lung dysfunction symptoms reveal as the destruction of cellular connections, increase in vascular permeability resulted in pulmonary edema, and infiltration of inflammatory cells. Neutrophils extend the organ injuries by secretion of some damaging substances like cytokines, granular enzymes, reactive oxygen species, and the organization of neutrophil extracellular traps (Fujishima, 2016). In this study, infusion of USSCs diminished the intra-alveolar infiltration of neutrophils and reduced lung edema and fibrosis in comparison with LPS-induced mice. Therefore, the cells may be a useful approach in moderating severe lung damage arising from the function of inflammatory cells.

Lung edema evaluated by wet/dry weighting arising from fluid effusion was followed by endothelial cell barrier destruction. The devastation of pulmonary endothelium barrier is frequently induced by LPS challenge in ALI animal model (Feng et al., 2013). USSC cells significantly declined the wet/dry ratio and consequently abnormal pulmonary edema 24 hr after administration. The ultimate therapeutic effect of USSC in pulmonary edema was notably along with MSCs-derived from the umbilical cord (Li et al., 2012) and MSCs-educated macrophages (Hu et al., 2016) that showed antiedema activity.

The liver is proposed as a critical organ in surviving patient with sepsis and related disorders because of detoxification functions. Liver damage is primed in the early stage of sepsis as a result of endotoxin and cytokine release, which is associated with some serological and histological change including severe increase of hepatic enzymes, hepatocyte apoptosis, necrosis, and neutrophil accumulation (Nesseler et al., 2012; Yan, Li, & Li, 2014). USSCs diminished liver injury in ALI mice by the reduction of hepatocyte necrosis, neutrophil infiltration, and vein dilation. On the other side, the decrease in the serum level of AST and ALT as the markers of hepatic damage may evidence the therapeutic effect of the cells on the liver dysfunction.

According to the results of this study, the xenotransplantation of USSC declined the mortality of LPS-challenged mice plus the effective recovery in cytokine releases and organ injuries. USSC secretome, containing various growth factors like angiopoietin-2, hepatocyte-derived growth factor (HDGF), transforming growth factor-β and macrophage migration inhibitory factor (Schira et al., 2015), may have a crucial effect in the treatment of a different aspect of sepsis disease. Furthermore, low immunogenicity, high proliferation, and nontumorigenicity, which is reported in the case of USSCs, are the main priorities in cell-based therapy. Our findings demonstrate the in vivo anti-inflammatory effect of USSC cells that may provide a path to use in human sepsis clinical trials.

In conclusion, we have investigated the therapeutic effect of USSCs derived from umbilical cord blood on the progression of LPS-induced ALI in which treated mice showed significant improvement in survival rate, reduction of systemic inflammatory responses, and moderation of organ injury. This study may provide promising results to introduce a notable cell source for the management of infectious disease to control inflammation and preserve the balance of immune responses via an immunomodulatory function.

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